THE OHIO JOURNAL OF SCIENCE

Vol. XXIII JULY–AUGUST, 1923 No. 4

THE COMPARATIVE PARASITIZATION OF CERTAIN FRESH WATER SNAILS.*

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INTRODUCTION.

This problem was begun at the Lake Laboratory in July, 1921, and continued in the Autumn and Winter of the same year at Ohio State University. It is essentially a comparative study of the parasites of certain fresh-water snails. The area from which snails were collected during the month of July was the region of the Bass Islands of Lake Erie. In order to compare conditions there with those of a stream, that part of the Olentangy River which runs through the property of the Ohio State University, was the place of collection in October and November.

While from the nature of the case some systematic work was necessary, the primary purpose of this study was bionomic. To determine the following, have been the aims of this investigation:

**A theisis submited in partial fulfillment of the requirments for the degree of Master of Science at Ohio State University.*

First, the species of fresh-water snails parasitized by larval trematodes;

Second, the identity of the trematode parasites;

Third, the approximate degree of parasitization in each case of infection;

Fourth, the percentage of parasitization within each species;

Fifth, the incidence of larval trematode infection among fresh-water snails.

The work was undertaken at the suggestion of Professor F. H. Krecker. Aside from his helpful suggestions I am indebted to Mr. Bryant Walker, of Detroit, for the identification of the snails; to a fellow student, Mr. James Davis, for a translation from the French of Dollfus' description of Cercaria pachycerca; and to the following for their previous studies on North American larval trematodes: Professors W. W. Cort, Johns-Hopkins University; E. C. Faust, Parasitologist, Union Medical College, Peking; and E. C. O'Roke, South Dakota State College. The literature on trematodes is cited, for no true internal parasites were found other than these.

HISTORICAL ACCOUNT.

"Among the early American Zoologists, Joseph Leidy alone was a student of Cercariæ."*[10]

In 1914, when W. W. Cort wrote his preliminary report: "Larval Trematodes from North American Fresh Water Snails," [4], he found eleven previous references, in North American scientific literature, to larval trematodes. In the last mentioned paper and in the monograph of 1915: "Some North American Larval Trematodes," [5] Cort described fourteen new species of Cercaria. The snails examined by Cort were from different localities throughout the United States, and from various ecological situations. The excretory system was taken as a conservative basis of description. Luhes scheme of classification was used. Not only was preserved material studied, but the reactions of live cercarial were noted. Cort, since then, has made several contributions to our knowledge of Digenetic larval trematodes.

In 1917, Earl C. O'Roke published a short paper on "Larval Trematodes from Kansas Fresh-Water Snails," [13]. This study was less pretentious than that of Cort's but added several new species.

*Numbers in brackets refer to the literature cited.

Also in 1917, Faust, in his preliminary report: "Notes on the Cercariæ of the Bitter Root Valley, Montana," [8] listed 32 larval trematodes which had been previously described. He described fifteen new forms. Faust's completed monograph, "Life History Studies on Montana Trematodes"[9] takes up the biology of a limited region, a detailed embryological and morphological study of the trematodes found there and their systematic relationship. Faust used not only the excretory system of live cercariæ as a basis of classification, but also worked out a method of staining the genital cell masses of the preserved material.

In 1919, Faust presented in "The American Naturalist," (No. 10, pages 85-92), a biological survey of cercariæ described from the United States up to that time. The total number of host records was 72. The net species was 61, as there were eleven species of larval trematodes recorded from two or more hosts. In 1921, the same author [11] described nine new larval distomes out of only two species of snails from the region of Rome, Georgia.

Other papers have appeared describing new species, clearing up developmental problems or group relationships, and some of the various phases in the irregular and complicated set of life histories. Despite the recent advances in parasitology, it is safe to say that only a limited area in the United States has been surveyed for larval trematodes. Also, the life-history and ecology of most of those named are only incompletely known. According to H. B. Ward [17, page 415], even the larva of the common sheep liver fluke, Fasciola hepatica, had not been reported from the United States up to 1918. Two years later, Mark F. Boyd, of the University of Texas, experimented to determine the intermediate host. He states [2]: "Data presented do not enable us to conclude that the larval cycle can be completed in Physa, nor settle the question regarding Linnæa humilis."

APPLICATIONS OF THE PROBLEM.

This last problem is of recognized economic importance in certain sections of the United States in connection with the live stock industry. Beside the fact that Fasciola hepatica is an occasional human parasite there are further reasons for a study of "flukes" and their host relationships. Cort[6] suggests the need of finding out what snails in this country may act as

intermediate hosts for the more serious trematodes which have already been introduced into this country by Orientals.

However, the immediate economic application of my study is to the problems of aquiculture—for example, to fish culture in Lake Erie and the streams of the state. At the same time and place that this investigation of the snail parasites was in progress, the fish parasites of Lake Erie were being studied by R. V. Bangham. During his work, my attention was called to his finding, in the digestive tract of the Small Mouth Bass, entire snails. Not only did this show that snails, which serve as food for fish, were a possible source of infection, but Digenetic trematodes were found as parasites of the fish.

F. C. Baker's ecological study [1, pages 153-154, 238-246], "The Realtion of Molluscs to Fish in Oneida Lake," shows the importance of a knowledge of snail parasites.

DEFINITION OF TERMS.

Out of the literature on trematodes certain terms have evolved. At this place it may be well to define some of the terms used throughout this paper.

According to Faust [9, pages 18 and 19], who follows Thomas (314), "the 'sporocyst' is the metamorphosed miracidium. It is merely a sac with endoderm covering and at times a secretory integument. Occasionally one end is partially muscular. From the inner wall of this sac arise the germ balls that grow into the parthenogenetic individuals. In the simplest types the germinal cell mass consists of the entire internal layers lying next to the ectoderm. In the majority of cases, however, the germinal tissue is localized at one end of the sporocyst. (C. dendritica Fig. 87, C. racemosa Fig. 105). In the furcocercariæ, C. gracillima and C. tuberistoma (Fig. 147, 157), there is a rhizoid-like attachment at the germinal end. In these cases there seems to be some evidence for regarding the germinal layer as localized at the end opposite the potential mouth."

Unless the rediac stage is omitted, it normally develops within the sporocyst stage. Faust states [9, page 20]: "Its organization is much more complex than that of the sporocyst. There is a well developed oral aperture, a muscular pharynx and a sac-like gut. There is a birth-pore just behind the collar region, on the left side, slightly ventral. Two projections, usu-

ally in the posterior part of the body, readily differentiate the redia externally from the shapeless sporocyst. In the cephalic region around the pharynx there is a nerve complex of highly differentiated nerve cells and nerve fibers. The integument is well developed and thick, and muscular layers within it play an important role in the movement of the redia, whereas the sporocyst depends almost entirely for its movement on the larva within it. In the mature redia the germ tissue is always localized at the posterior extremity of the body."

In another connection Faust states [10, page 92] "Sporocysts and rediæ have not been sufficiently distinguished. The sporocyst is an adult which has lost its digestive tube, while a redia is an adult which possesses both a rhabdocœle gut and a pharyngeal sphincter. In certain sporocysts the sphincter still remains, as in C. dendritica. In other sporocysts, as in furcocercariæ, while no definitely differentiated sphincter is present, the anterior end of the sac is muscular, turning in and out like the finger of a glove. This may be mistaken for a rhabdocœle gut."

According to Faust [9, page 14], Ssinitzin groups the mature sporocysts and redia together under the term "parthenita," or parthenogenetic mother. The term is used throughout this paper to define the mature sporocysts and redia as distinguished from cercaria or other larval trematode of the hermaphroditic generation."

PROCEDURE.

In this study of comparative parasitization, the snail hosts will first be considered. Representatives from all the available fresh-water snails were collected, in July from the Bass Island region, and in October and November from the Olentangy river. The various snails were collected from a number of places in these regions, whenever their range of distribution was not limited to a definite ecological situation. Representative shells from the lots examined were identified by Mr. Bryant Walker, of Detroit. These were the lake snails examined: Limnæa humilis, Goniobasis livescens, Physa ancillaria, Planorbis parva, Planorbis trivolvis and Pleurocera acuta. The river snails were: Limnæa humilis, Ancylus rivularis, Goniobasis livescens, Physa ancillaria and Planorbis trivolvis.

A few snails were collected at a time and examined as soon as possible thereafter. The examination in each case consisted

of first crushing the snail or removing the shell piece by piece from the body. Then the snail was placed in a watch glass in a small amount of water. Only enough water was used to allow movement of parasites when freed from the snail tissue. Under a binocular microscope the body of the snail was teased apart with needles. If no parasites were detected in a given snail, the watch-glass in which the examination was made, was washed out before examining another snail. If trematode parasites were present in a given snail, they could be detected both by general appearance and by movements, often characteristic for different trematodes. These peculiarities, as to reactions of live cercaria, as well as the relative number of parasites present were noted. Each lot of parasites from a single snail, were fixed in Hofer's solution and preserved in alcohol for later study. Every lot was kept in a bottle labeled with the serial number of the snail from which it came. In this way the number of snails examined from each species, the number parasitized in each species and the approximate infection of any snail examined was recorded.

The arbitrary terms used for this last item were: "few" for less than ten parasites, "many" for from ten to about one hundred and "very many" for the heaviest infections.

After examining 1043 snails representing the seven species previously named, attention was turned to a study of the trematode parasites obtained from them. From the preserved material, "toto" mounts were made of both parthenitæ and larvæ (cercariæ). These were studied under the compound microscope. Measurements were made of cercariæ and parthenita. The parthenita stage, whether sporocyst or redia, accompanying the various cercariæ was noted. From the literature that is now available, identifications were made. Ward's "Key to North American Trematoda" [17, pages 411–424] was supplemented by one of his shorter papers, the more complete descriptions in the monograph by Cort [5] and Faust [9], and the paper by O'Roke [13].

DATA IN TABLE FORM.

In the tables that follow is presented a comparative study of the parasites found in this investigation. The exact number of snails examined, the ones parasitized and observations as to the parasites are recorded.

SNAILS FROM REGION OF PUT-IN-BAY, OHIO, COLLECTED JULY, 1921.

Host	Location	Number Examined	Parasitized		Serial Number	Name of Parasite	Kind	Parthenita		Cercaria (larva)
			Number	Percent				Sporocyst	Redia	
Limnaea humilis	Squaw									
	Harbor	100	0	0						
Physa	Chicken I.				304		Distome			√
ancillaria	Buckeye I.				338	(Lost)				Few
ancillaria	Hatchery				358		Distome			√
ancillaria	Bay				364	C. urbanensis	Monostome		√	√
ancillaria	Bay				379	C. urbanensis	Monostome		√	√
ancillaria	Chicken I.	100	6	6	397	C. gracillima	Distome			√
Planorbis	Chicken I.				208	(Lost)				
parva	H. Bay				255	C. urbanensis	Monostome			Many
parva	H. Bay				275	C. urbanensis	Monostome		Few	Few
parva					275		Distome			
parva	Sq. Harbor				297	C. urbanensis	Monostome		Few	
	Sq. Harbor	100	5	5	298	C. urbanensis	Monostome		Few	
Planorbis trivolvis	Sq. Harbor	46	1	2.17	640	C. echinocauda	Distome	√		√
Pleurocera	Sq. Harbor				4					√
acuta	Sq. Harbor	100	2	2	7			√		√

SNAILS FROM REGION OF PUT-IN-BAY, OHIO, COLLECTED JULY, 1921—Continued.

Host	Location	Number Examined	Parasitized		Serial Number	Name of Parasite	Kind	Parthenita		Cercaria (larva)
			Number	Percent				Sporocyst	Redia	
Goniobasis	Hatchery				501	C. microcotylae				
livescens	Bay				502	" "	Distome			Many
"	"				509	" "	"			√
"	Hen Island				512	C. anchorcides	"			√
"	Hatchery				514	C. microcotylae	"			√
"	Bay				532	" "	"			√
"	"				545	" "	"			Many
"	"				549	C. urbanensis Cort. 1914	Monostome			√
"	"				"	C. microcotylae	Distome			√
"	"				556	" "	"	Few		Few
"	"				557	" "	"	Few		Few
"	"				559	" "	"			√
"	"				560	" "	"			√
"	"				565	" "	"			Few
"	"				567	" "		Very many		√
"	"				568	" "	"			√
"	"				574	" "	"	Very many		√
"	"				576	" "	"			Very many
"	"				581	" "	"	Many		√
"	"				595	" "	"			Few
"	"	100	19	19	599		"	Few		Few

SNAILS FROM OLENTANGY RIVER, COLUMBUS, OHIO, COLLECTED OCT.-NOV., 1921.

Host	Location	Number Examined	Parasitized		Serial Number	Name of Parasite	Kind	Parthenita		Cercaria (larva)
			Number	Percent				Sporocyst	Redia	
					1432	(Lost)				Few
Limnaea humilis	Olentangy Riv.	100	2	2	1432	(Lost)				Few
Ancylus rivularis	"	100	0	0						
Goniobasis livescens	"				904	C. pachycerca				
" "	"				905	" "	Distome	Few		
" "	"				"	C. microcotylae	"	"		Many
" "	"				910	" "	"			
" "	"				938	C. megalura	"	"		"
" "	"				962	C. gracillima	"		Many	"
" "	"				963	" "	"	"		"
" "	"	86	7	81	965	C. megalura	"	Many		Very many
Physa ancillaria	Olentangy Riv.				703	Curbanensis	"		Many	Many
" "	"				801		Monostome		Few	Many
" "	"				804				Few	Few
" "	"				812	(Lost)?			√	√
" "	"				815	(Lost in clearing)				Few
" "	"				816	(Lost)?	Distome		Few	Few
" "	"				822					1
" "	"				838		Distome		Few	Very many
" "	"				1302	(Lost)?			Many?	
" "	"	200	10	5	1372	(Lost)?				√
Planorbis trivolvis	Olentangy Riv.	9	0	0						Many

COMPARATIVE PARASITIZATION SUMMARY.

Species of Snail Host	Location of Snail Hosts	Number Examined	Parasitized		Species		Stages	
			Number	Percent	Monostome	Distome	Parthenita	Cercaria
Limnaea humilis	Lake Erie	100	0	0				
Limnaea humilis	Olentangy River	100	2	2		√		√
			(2)	(1)				
Ancylus rivularis	Olentangy River	100	0	0				
		100	20	20	1	√	√	√
Goniobasis livescens	Lake Erie	86	7	8.1		√	√	√
Goniobasis livescens	Olentangy River		(27)	(14.5)				
		100	6	6	1	√	√	√
Physa ancillaria	Lake Erie	200	10	5	1	√	√	√
Physa ancillaria	Olentangy River		(16)	(5⅓)				
		100	5	5		1	√	√
Planorbis parva	Lake Erie	46	1	2		1		
Planorbis trivolvis	Lake Erie	9	0	0				
Planorbis trivolvis	Olentangy River	100	2	2		√	√	√
Pleurocera acuta	Lake Erie	1043	53	5.03				

DISCUSSION.

The data here presented in table form indicates that seven species of fresh-water snails have been examined for parasites. Of these, six species are shown to be parasitized by Digenetic larval trematodes. The incidence of larval trematode infection of fresh-water snails is shown. From 1043 specimens examined, 53 snails, or more than one in every twenty were parasitized by some stage of Digenetic trematode. Of the 53 host snails, thirty are represented by mounted material, ("toto mounts"). Instead of redescribing the identified parasites, the source of the original description is given. Some variations or additional facts are noted.

Cercaria urbanensis Cort 1914, was the only species of monostome found. Either the parthenita or larva stages of this parasite were found in seven snails. The hosts in which this specie was found were: Physa ancillaria in one snail each from the river and lake; Planorbis parva in four out of 100 Lake Erie snails; and a lake specimen of Goniobasis livescens. The morphological characteristics fit the description for Cercaria urbanensis given by Cort [5, pages 11-17) and in Ward's Key [17, page 412]. Part of Cort's record of infection is quoted [5, page 11): "About five percent of the largest specimens of Physa gyrina from a drainage ditch North of Urbana, Illinois, examined in December, 1913, were infected with the redia and cercaria of a monostome. The infection was in the liver of the snail and there were present both rediæ in different stages of development and free cercariæ."

Of the distome cercaria, there were several kinds. Nineteen percent of the lake specimens of Goniobasis livescens were heavily infected by small distome cercariæ. Both the larva and parthenita stages respectively were very similar in all of these small distomes. They are very closely related to Cercaria leptocantha, a Xiphidiocercaria described by Cort [5, pages 59-62; figures 79-81]. He says of this species (5, page 60-61): "Cercaria leptocantha belongs to a group of very small cercariæ which Luhe [12, 1909, page 106] calls Cercaria Microcotylæ. It is possible that they form a natural group. * * * They are, however, so insufficiently known that no final judgment can be passed on their relationships. At present it seems best to follow Luhe in considering them a provisional

group, with Cercaria microcotyla Fillippi as the type and the following characteristics.

1. Developed in gastropods in round or oval sporocysts which are seldom more than twice as long as wide.
2. Cercaria under 0.2 mm. in length.
3. Acetabulum back of the middle of the body and smaller than the oral sucker.
4. Stylet glands not more than four on each side and arranged in rows on each side of the acetabulum.
5. Digestive system undeveloped except for a short prepharynx and a small pharynx."

A much larger distome, a gymnocephalous cercaria closely related to the cercaria of Fasciola hepatica is identified as Cercaria (Gymnocephala) ascoides Leidy 1877. It was found in a specimen of Planorbis parva from Lake Erie. Cercaria urbanensis was found in the same snail. This is a case of double infection, one parasite being a monostome and the other a distome. There were only two of these relatively large distomes. It may be noted in this connection that there seems to be little relation between the size of the snail host and the trematode parasite. The smallest cercaria, as well as the largest, was found in Goniobasis livescens. Goniobasis livescens is one of the largest of the seven snails examined, being many times the size of Planorbis parva, which is the smallest of these snail hosts.

The other species of distome, from a lake specimen of Goniobasis livescens, was Cercaria anchoroides. This peculiar cystocercous cercaria was described by Ward [15] from free-living specimens which he found in Lake St. Clair between July 27 and August 5, 1893. In 1916, he again described this cercaria, [16], but reported no host for it. On July 17, 1921, from snails taken in Lake Erie off the shore of Hen Island, I found not only a cercaria in the characteristic anchor-like stem, or tail which pulls the cercaria along, but also sporocysts. In one of these brown colored sacs, the inverted flukes and anchor stem were protruding at right angles to the sporocyst wall. In another case, the forked tail or wings of the anchor could be seen inside the sporocyst. The reversal of functional activity in the tail of the live cercaria, the wavy outline of the intestine and the proportion of parts indicate that the larval trematode found near Hen Island is of the same species as that described by Ward from

Lake St. Clair. The host of this peculiarly specialized cercaria is for the first time recorded as Goniobasis livescens.

At least three species of the distomes were furocercariæ, if the large cystocercous larva, Cercaria anchoroides, is included. One of these forked-tailed cercariæ was concluded to be Cercaria echinocauda described in 1817 by O'Roke, [13, pages 170-171; figures 39-43). He states, [3, page 170): "Six percent of large numbers of Physa gyrina collected at Lakeview, Kan., August 20 were infected with a form for which I propose the name Cercaria echinocauda. Collections of snails from the same locality made October 12, showed two percent to be infected." In this investigation, the host was Planorbis trivolvis from Lake Erie. Only one snail was infected by this parasite. O'Roke's description of Cercaria echinocauda fits for this parasite, with the exception of his Figure 39 on Plate V. That figure shows the large pigmented eyespots to be set with their long axis at about a forty-five degree angle to the long axis of the body, with the anterior ends of the eyespots farther apart than the smaller posterior ends. A camera lucida drawing with the 4 mm. objective of the parasite from Planorbis trivolvis, which agrees in other respects to the one O'Roke described, shows definitely that the eyespots are set at right angles to the direction which his drawing represents. The anterior ends of these eyespots are directed toward a point which would be midway between the region of the eyespots and the extreme anterior end of the body.

Several specimens of a forked-tailed cercaria, found in a specimen of Physa ancillaria from the Olentangy river, were lost in clearing.

The other species of furcocercariæ was found twice in Goniobasis livescens from the Olentangy river and once in Physa ancillaria from Hatchery Bay, Lake Erie. Faust's description for Cercaria gracillima (9, pages 80-82; figures 142-154), from Montana more nearly fits the parasites from these three specimens than any other description that I have seen. According to Faust [10, page 91]: "None of the species described for the Bitter Root Valley have been recorded east of the Rocky Mountains." This may only indicate the small geographical area that has been covered, for, as he suggests on the same page the same condition is not true in general for other larval trematode parasites. The following is the record of parasitization as given by Faust [9, page 81]: "This species was found in the liver of Physa

gyrina Say, collected from the lower reaches of the Bitter Root River near Maclay Bridge, Buckhouse Bridge, and the sloughs at the roadhouse, near Fort Missoula, in the fall of 1916. In addition, it was found in the livers of Limnaea proxima Lea from Rattlesnake Creek, Missoula. The infection in most cases was not exceedingly heavy, except in the collection from the sloughs at the Roadhouse, where thirty-three out of seventy-one individuals were infected, or 46.5 percent.

Out of the 87 specimens of Goniobasis livescens from the Olentangy River, seven were infected. Of these, two infestations were with microcotylous cercariæ similar to those from the lake specimens of Goniobasis.

In two of the 87 snails there were larvæ identified as Cercaria grocillima Faust 1917.

Two other individuals of Goniobasis livescens from the river were infected with Cercaria megalura Cort 1914. [5, pages 31–36; figures 27–36]. Beside the group of gland cells forming an adhesive organ in the tip of the contractile tail, the shape, size and other characteristics make this identification very certain.

Also, different specimens of the mounted parasites showed, as Cort's figures do, a varying amount of the cytogenous material within the body or thrown out around it. As to the classification, Cort removed this trematode from the subdivision of Gymnocephalous cercaria. He calls it a megalurous cercaria, from the characteristic appearance of the tail. As to the parasitization of his material Cort states [5, page 31]: "From 73 specimens of Pleurocera elevatum from Sangamon River near Mahomet, Illinois, examined during November and December, 1913, one was found with the liver packed with redia in which developed a very peculiar kind of cercaria. Comparison showed this form to be the same as the larger cercaria which Cary assigns to Diplodiscus temporatus." Cary's snail host was Goniobasis virginica Say from Princeton, New Jersey (1908). He left no record as to the extent of parasitization.

In two specimens of Goniobasis livescens from the Olentangy River were cercariæ which to my knowledge have not been described from North America. They differ from Cercaria trigonura Cort 1914, [5, pages 49–52; figures 47–54], in their stumpy tails which are truncate, nearly as broad as long and the width about the same at each end. The tail of a represen-

tative cercaria measured 0.066 mm. long by 0.055 mm. wide. The cup-like end functions as a sucker rather than the ventral part of the triangular shaped tail of Cercaria trigonura. The bladder is large and entire as contrasted with the bicornuate excretory vesicle of Cercaria trigonura. It extends above the acetabulum occupyihg almost the posterior two-fifths of the body. Aside from the tail and size, the general appearance of the body of this cercaria is similar to that of Cercaria trigonura.

These distome cercariæ from Goniobasis livescens average about 0.4 mm. in length by 0.1 mm. in width. Cercaria trigonura Cort 1914 [5, page 45] is stated to measure 0.24 mm. in length and 0.06 mm. in width. Further, their parthenitæ differ both in size and kind. Cercaria trigonura develop in rediæ measuring 0.43 mm. in length and 0.09 mm. in diameter [5, page 47]. The stumpy-tailed cercaria from Goniobasis livescens develops in sporocysts like the Cotylocercous cercariæ of Dollfus [7]. While the size of the parthenita, as well as the cercariæ varied slightly, the relative size of the two is shown by these measurements taken respectively from an average sized cercaria and a sporocyst mounted together: cercaria 0.4 mm. by 0.1 mm. and sporocyst 2 mm. in length by 0.5 mm. in width.

Robert Dollfus in his preliminary report at the Ninth International Congress of Zoology held at Monaco in 1913 [7, page 684], says:* "The group of Cotylocercous cercaria, with the common type Cercaria pochycerca Diesing, are characterized as follows: a large bladder, not bifurcate, occupying nearly all of the posterior part of the body. Beginning with the ventral sucker its wall is formed of a single layer of very large closely fitted granular cells of glandular appearance. Tail very short, large at least at its base, consisting of a cup with very thick wall and large cells. The bottom of the cup and the sides by their combined movement permit of adhesion to a support after the manner of a sucker. This tail, functioning as a sucker, is well developed."

These stumpy-tailed cercariæ from Gonibasis livescens are identified as belonging in the group of larval trematodes which Dollfus calls Cotylocerous, with Cercaria pachycerca Diesing as the type species.

Some observations as to the ecology of this and other larval trematodes may be in place. Reference has already been

*Translated from the French by James E. Davis, O. S. U.

made to the apparent lack of relation of the size of the snail host to that of the parasite. It will be observed from the tables that Microcotylous cercariæ were the predominant parasites of the lake specimens of Goniobasis. In the individuals of Goniobasis from the Olentangy River, there were no Monostome cercariæ, but there was a wider range in the type of distome larvæ. There was a double infestation at each place.

From the river, two individuals were infested with Cercariæ microcotylæ (very small distomes), two with Cercaria pachycera (a stumpy-tailed larva), two with Cercaria gracillima (a forked-tailed cercaria with eye-spots) and two with Cercaria megalura with the characteristic group of cells for attachment in the end of the long tail. Other particulars might be stated as to the parasites and the environment of the snail hosts. However, the general conclusions of Dollfus as to the ecology of larval trematodes is borne out by this study of their comparative parasitization.

As to the relation of the morphology of the trematode parasites to the environment Dollfus states [7, page 685]: "After comparative examination of the morphology of the cercaria and the condition in which the host lives I have been able to submit that cercariæ with a morphology very similar have hosts very different and lives very different. Cercaria very different inhabit the same environment ("Milieu"). I have been able to establish that it is only arbitrarily that one attributes certain forms to certain places."

Faust [10] states in this connection that, "The mollusks most heavily infected are the ubiquitous species, Planorbis trivolvis and Physa gyrina, and the western species Lymnea proxima.

Beside calling attention to the limited geographical areas that have been covered in the survey for cercaria Faust suggests that [10, page 91]: "the variation of species of flukes in snails from one season to another makes it highly probable that many more species occur in the mollusca of the areas surveyed than the records show."

SUMMARY.

The comparative parasitization of seven species of freshwater snails, totaling 1,043 specimens, has been studied. The species were Limnæa humilis, Ancylus rivularis, Goniobasis

livescens, Physa ancillaria, Planorbis parva, Planorbis trivolvis and Pleurocara acuta.

Six of these species have been shown to be parasitized by the larval stage of Digenetic trematodes. No parasites were found in the 100 specimens of Ancylus rivularis.

The kinds of cercaria identified were monostomes: (Cercaria urbanensis Cort 1914);
Distomes:

Microcotylous, having as type species (Cercaria leptocantha Cort 1914).

Furcocercous, (Cercaria echinocauda O'Roke 1917),
(Cercaria gracillima Faust 1917).

Cystocercous, (Cercaria anchoroides Ward 1916).

Megalurous, (Cercaria megalura Cort 1914).

Stumpy tailed or Cotylocercous, (Cercaria pachycerca Diesing).

The approximate degree of parasitization of individual snails has ranged from none, (in Ancylus rivularis), to several hundred, (in the case of Goniobasis livescens parasitized by Cercariæ microcotylæ).

There were two cases in which two species of trematodes were found in a single host. In one of these cases, one parasite was a monostome and the other a distome larva.

Little, if any, relation was found between the size of the host and the size, or number of its parasites.

The percentage of parasitization found for the seven species of freshwater snails was as follows:

Goniobasis livescens	14.5	percent
Physa ancillaria	5.33	"
Planorbis parva	5.0	"
Pleurocera acuta	2.0	
Planorbis trivalvis	2.0	"
Limnaea humilis	1.0	"
Ancylus rivularis	0.0	"

The incidence of larval trematode infection among certain fresh-water snails has been shown to be over five per cent.

A host, Goniobasis livescens from Hen Island, Lake Erie, was found (July 17, 1921) for Cercaria anchoroides previously only recorded as free-swimming.

A stumpy tailed distome Cercaria pachycerca Diesing, has been reported as a new species from North America.

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Recieved for publication, January 18, 1923

CHEMICAL COMPOSITION OF THE WALLS OF CERTAIN ALGAE.*

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INTRODUCTION.

In spite of the fact that numerous investigations of the nature of cell walls of algæ have been made, the recorded data are still far too incomplete to be of the greatest service to science. It is usually impossible to obtain from the literature a complete record of the composition of the walls of the most commonly occurring fresh water algæ. The physiological nature of the cell walls of algæ appears to play an important role in such phenomena as the resistance of certain algæ to desiccation during dry seasons, the rate of entrance of certain mineral elements, the attachment or nonattachment of epiphytic algæ, and the utilization of algæ as food by fishes and other aquatic animals. It seems important that a thorough knowledge of the composition of the cell walls of algæ should be obtained. The object of the investigation reported below was to determine the composition of the cell walls of some of our common algæ. Representatives of the following genera were studied: *Vaucheria*, *Cladophora*, *Oedogonium*, *Spirogyra*, *Zygnema*, and *Draparnaldia*.

LITERATURE.

Only those papers dealing with investigation of the genera listed above will be reviewed in this paper. In 1913, Mirande (4) reported that the cell walls of *Vaucheria* were composed entirely of cellulose-pectic compounds. He did not state whether these compounds were intermingled or in separate layers, or in what form the pectic compounds occurred.

Brand (1) states that stratification of the cell walls of *Cladophora glomerata* begins in a very early stage, young filaments of two cells being composed of two layers. The outermost layer surrounds the entire filament and is loosened from

*Papers from the Department of Botany, Ohio State University, No. 141.

it by dilute acetic acid or other weak acids. Potassium hydroxide, alcohol, and acid free glycerine have no effect on it. This membrane appears to be independent of either of the other two membranes constituting the second layer of the cell wall. It gave no positive reactions for cellulose. The cellulose reaction was obtained with difficulty, so that it seemed that the pectic compounds predominated in the membrane. Chlorzinc iodide gave no blue color even after boiling with hydrochloric acid.

Hirn (3) found the cell walls of Oedogonium to be composed of two layers. The inner one was a moderately thick layer of cellulose and the other a "cuticle" which enclosed the entire filament. This "cuticle" could not be identified with that of the higher plants by means of either stains or other reagents.

Hirn also reports (3) that the beginning of the new partition wall in Oedogonium was at first a thin cellulose ring. This ring enlarges and appears stratified, and separates into two entirely different regions. The outer one of these regions gives a positive cellulose reaction and the inner one a negative reaction for cellulose. Klebahn says [according to West (7) and Oltmanns (6)] that the ring is of a gelatinous nature and stains with hæmatoxylin. The young membrane which comes from the ring stains in the same manner while the older membranes do not.

Czapek (2), in his discussion of the cell walls of the green algæ, states that Klebs found the wall inside the gelatinous sheath of *Zygnema sp.* to have no homogeneous composition. Part of the membrane, which in the normal cell membrane stained with the aniline dyes, was dissolved by heating in dilute hydrochloric acid. The remainder of the cell wall was readily dissolved in copperoxide ammonia, stained with congo red and gave the usual cellulose reactions.

West (7) reports all the members of the group Conjugatæ to have a firm cellulose wall and some to have a great development of the mucilaginous pectose constituents of the cell wall. This development may be through the conversion of the outer cellulose layers into mucilage or by mucilage being continually exuded through the cell wall.

METHODS OF INVESTIGATION.

All the filaments were mounted in distilled water and examined in polarized light. The results of these data were then checked by color reactions, hydrolysis, and solubility of the membranes.

The following tests were used in the identification of the substances found:

1. Cellulose. Cellulose is doubly refractive in polarized light, stains blue with iodine potassium iodide and 70% sulphuric acid, and is soluble in copperoxide ammonia. The filaments are left intact when the cellulose has been removed and will then give a negative hydrocellulose reaction and show no double refraction in polarized light.

2. Pectic Compounds. All pectic compounds are singly refractive in polarized light. The different compounds are distinguished by their different solubilities. Pectic acid is soluble in 2% potassium hydroxide or in 5% sodium carbonate, and is dissolved from the filament by treating it with either of these reagents. If the filament still shows the presence of a pectic compound it is then treated for the removal of calcium pectate.

Calcium pectate is broken down by 2% oxalic acid, giving calcium oxalate crystals and pectic acid. The calcium oxalate crystals may be recognized by their tetragonal shape and their double refraction of polarized light. The pectic acid may be removed by dissolving it in 2% potassium hydroxide or 5% sodium carbonate as stated above.

Any pectic compound remaining is pectose and is dissolved by heating the filament on the water bath 20—30 minutes in 2% hydrochloric acid and then treating with 2% potassium hydroxide. The hydrochloric acid breaks the pectose down to pectin or pectic acid. If to pectin, the compound is removed by washing the filament in water. If to pectic acid the potassium hydroxide dissolves it.

If fresh untreated tissue is used in making these tests, the cells are left intact after all the pectic compounds have been removed. These cell walls will then react negatively for pectic compounds in polarized light, but positive by for any other substance composing the walls.

3. Chitin. Chitin, when present, seems to be in a thin layer on the outside. It is singly refractive and can not be distin-

guished from the pectic compounds in polarized light. Tissues containing chitin give a positive chitosan reaction. Filaments of the alga are placed in boiling concentrated (50%) potassium hydroxide, and allowed to boil in a covered dish for 30 minutes. The filaments are then hardened in 90% alcohol, mounted in iodine potassium iodide, and dilute sulphuric acid added. A red violet color, characteristic of chitosan, is obtained.

REPORT OF INVESTIGATION.

VAUCHERIA GEMINATA. In polarized light the cell walls of *Vaucheria geminata* are composed of two layers, the outer one being singly refractive and the inner one doubly refractive. This outer layer gives positive reaction to all tests for pectose. Other pectic compounds, if present, occur only in small amounts. Before treating with the pectose solvents the inner layer reacts negatively to the hydrocellulose reaction, although it is dissolved in copper oxide ammonia if left in the solution two days, the solution being changed three times. After the removal of the cellulose the filament, now composed only of pectose walls, is left intact although very fragile. After removing the outer layer of pectic compounds, the inner layer of the cell wall reacts readily to the cellulose reagents, giving a positive test to the hydrocellulose reaction and dissolving rapidly on the slide in copperoxide ammonia. The entire cell wall is dissolved when treated with the solvents for both pectic compounds and cellulose.

The mature oospore seems to have a layer in addition to the two present in the cells of the filament. A thin membrane is left intact after both the pectic compounds and the cellulose have been removed. This membrane is broken by the pressure of the cell contents while the swelling cellulose is being dissolved. The cell contents move out of the oospore wall leaving it intact as an empty shell. This shell is singly refractive. It may be chitin but the exact composition has not been determined.

To summarize the work on *Vaucheria geminata:* the cell walls are composed of pectose and cellulose, the former being on the outside. This outer pectic layer is very difficultly permeable to cellulose reagents, particularly those used in the hydrocellulose reaction, presumably iodine, though this point was not

definitely determined. The mature oospore has an additional outer layer which may be chitin.

CLADOPHORA GLOMERATA. The cell walls of *Cladophora glomerata* are composed of three layers. The outermost layer is of chitin and can be separated from the rest of the filament by dilute acetic acid. This layer is singly refractive in polarized light, insoluble in either pectic or cellulose solvents, and gives a positive chitosan reaction.

The second layer is also singly refractive in polarized light but is soluble in the pectose solvents. The inner layer is doubly refractive in polarized light but gives the characteristic cellulose reactions only after the filament has been treated with pectose solvents. Then the cellulose is readily dissolved leaving only the outermost layer of chitin.

Therefore, the cell walls of *Cladophora glomerata* are composed of three layers: chitin, pectose, and cellulose. The layer of chitin is readily separated from the rest of the cell wall and is the only membrane left intact after the filaments have been treated with pectose and cellulose solvents. This layer is also evidently very difficultly permeable to cellulose reagents until partially hydrolyzed. It appears to be more impermeable to copper than pectose is. It is interesting to note in this connection that Moore and Kellerman (5) find that the per cent of copper sulphate required to destroy Cladophora in reservoirs is twenty-five times that required to kill Spirogyra which has no chitinous membrane.

OEDOGONIUM IRREGULARE. The young filaments, of from one to two cells in length, of *Oedogonium irregulare* give no reaction for the presence of cellulose. They are composed of two layers, the outer one of which may be separated from the other and be made to bulge out by dilute acetic acid. This layer gives the chitosan reaction and is singly refractive in polarized light. It surrounds the entire filament but does not form a part of the cross cell walls. The inner layer of the wall is of pectose and may be dissolved by heating in 2% hydrochloric acid for one-half hour and then leaving in 2% potassium hydroxide for 15 minutes or longer. The outer layer is left intact after this treatment.

When the filament is older and is composed of two or more cells, a third layer appears inside the layer of pectose. This

layer is doubly refractive, but gives the cellulose reactions only after the filament has been treated for the removal of pectose. The layer of chitin is evidently partially hydrolyzed by this treatment since a slight chitosan reaction is also obtained when applying the hydrocellulose test. This layer of chitin and the layer of pectose are undoubtedly the membranes which prevent the entrance of copper and iodine into the cell when tests are made for cellulose and may explain why it has been generally considered that the cell walls of water plants were composed of difficultly soluble cellulose. In these investigations it was found that when these impermeable layers were removed or hydrolyzed the cellulose in the walls of algæ is no more difficultly soluble than that in higher plants.

Oedogonium crassum amplum. The walls of the vegetative cells of *Oedogonium crassum amplum* are stratified and composed of three substances. The outer layer surrounds the filament as a whole and can be readily separated from it by treatment with copperoxide ammonia. When this solution is run under the coverglass the outer membrane swells and bulges out from the other membranes, but does not dissolve. It is not caused to separate from the filament when treated with acetic acid as was the case in *Oedogonium irregulare*. It is singly refractive and, although it is soluble in neither pectose nor cellulose solvents alone, it is finally dissolved with the rest of the cell wall when both solvents are used. It apparently is not a mixture of cellulose and pectic compounds since it does not give a positive reaction for celluolse when the pectic compounds are removed. It gives a negative reaction for pentosans.

The second layer of the cell wall is of pectose and is dissolved in hydrochloric acid and potassium hydroxide. This membrane is singly refractive in polarized light.

The inner layer is doubly refractive and gives the characteristic hydrocellulose reaction for the presence of cellulose. When the sulphuric acid is added in the hydrocellulose reaction, the cellulose membrane seems to loosen at one end of the cell and expand, remaining attached at the other end. In the same reaction the filament undergoes a series of color changes before the final blue color is attained. The filament becomes red and then goes through violet to the characteristic deep blue color of cellulose. After being treated with the pectose solvents, the

filament is stained red directly with iodine potassium iodide, then changes to blue when 60% sulphuric acid is added. These peculiar color changes indicate the presence of some unidentified substance in the walls of this alga.

When the sulphuric acid or copperoxide ammonia is added the cells separate from each other, the cellulose layer swells rapidly becoming many times its original thickness, then it divides into two or more parts forming distinct cylinders which rapidly slip out of each other before going into solution. The entire filament is soluble in copperoxide ammonia after the pectic compounds have been removed. The pectose solvents evidently partially hydrolyse the outermost layer which may be composed partly of some form of chitin or a hemicellulose.

The ring, which is the first indication of the new partition wall in cell division, is singly refractive in polarized light, indicating that it is not pure cellulose. It is insoluble in either the pectic solvents or the cellulose solvent when they are used alone, but when the filament is treated with both groups of solvents the ring is dissolved as well as the rest of the cell wall. It gives a faint blue when treated with the hydrocellulose reagents, but gives a negative test for cellulose under polarized light both before and after treatment with pectose solvents.

To sum up, the cell walls of *Oedogonium crassum amplum* are striated and are composed of three substances: an unidentified substance on the outside, which can be separated from the rest of the filament; a middle layer of pectose; and an inner layer of cellulose. The ring formed in the dividing of the cells may be in part a mixture of pectose and cellulose, though it resembles rather closely the unidentified outer layer of the filament.

Spirogyra fluviatilis. The cell walls of this Spirogyra are composed of two layers. The outer layer is of pectose, being singly refractive in polarized light and dissolved from the wall by 2% hydrochloric acid followed by 2% potassium hydroxide. The inner layer is of cellulose, but unlike that of Vaucheria, Cladophora, or Oedogonium, it reacts readily to both the hydrocellulose and solubility reactions for cellulose before any previous hydrolysis of the outer membranes of the cell walls. This indicates that the pectic compound present in *Spirogyra fluviatilis* is not as impermeable to copper and iodine as in the

other cases, and may explain why Spirogyra is so readily killed by copper sulphate as found by Moore and Kellerman (5).

In the formation of the conjugation tubes there is a gradual change in the composition of the walls as they increase in age. During the slight bulging of the vegetative cell wall, which is the first indication of the formation of the conjugation tube, there is no obvious alteration in the original cell wall. When the tube is completely formed, the cellulose extends only a short distance into the tube, the rest of the wall being composed of a single layer of pectose. In a more mature conjugation tube of another species it was noted that the cellulose lined the entire tube, making the cell wall of two layers, the outer one of pectose and the inner one of cellulose as in the wall of a vegetative cell.

To summarize, the cell walls of *Spirogyra fluviatilis* are composed of two layers; the outer one is of pectose and is readily permeable to reagents containing either copper or iodine; the inner layer is of cellulose and gives all the characteristic cellulose reactions. In the conjugation tubes the cell wall is at first composed of a single layer of pectose, a layer of cellulose being formed later. Four other species of Spirogyra examined showed the same cell wall structure as described above.

Zygnema cruciatum. The cell walls of *Zygnema cruciatum* are composed of three layers. Around the entire filament is a thick mucilaginous sheath of pectic acid. This sheath is singly refractive in polarized light and is dissolved in 2% potassium hydroxide. Just inside the mucilaginous sheath is a more compact layer of pectose which may be removed from the filament by treatment with the pectose solvents. The innermost layer of the cell wall is left intact after the outer layers have been dissolved. This layer gives the characteristic cellulose reactions, being doubly refractive to polarized light, positive for hydrocellulose, and soluble in copperoxide ammonia. These reactions are positive both before and after the pectic compounds have been removed, indicating that the pectic compounds in this species are also readily permeable to copper and iodine.

Draparnaldia plumosa. The filaments of *Draparnaldia plumosa* are surrounded by a thick slimy sheath. This slime is singly refractive in polarized light and is partially soluble in 2% potassium hydroxide, more soluble in 5% sodium carbonate,

and entirely soluble in the pectose solvents, indicating that it is composed of both pectic acid and pectose.

The cell walls of the main filaments are composed of two layers. The outer layer is of pectose and the inner one of cellulose. Before the slimy sheath has been removed, the cellulose membrane gives a negative reaction to the hydrocellulose test. After the treatment with pectose solvents, the cell walls of the main filament give a positive reaction to the hydrocellulose reagents. The cell walls of the branch filaments are singly refractive in polarized light and give no hydrocellulose reaction, but when the filaments have been left in the potassium hydroxide of pectose solvent for several hours, they are dissolved, with the cell walls of the main filament, in copperoxide ammonia.

The above data seem to indicate that the main filaments of *Draparnaldia plumosa* are composed of cellulose and pectose in layers, while the branch filaments are of a combination of cellulose and a pectic compound. Of these two substances the pectic compound seems to predominate, masking the polarized light reaction of the cellulose present. The entire filament is surrounded by a slimy sheath of pectic acid and pectose. This sheath prevents the cellulose reagents from coming in contact with the cellulose, thereby preventing a positive test for cellulose until the slime has been dissolved.

TETRASPORA. The mucilaginous sheath surrounding the colonies of Tetraspora is singly refractive in polarized light and is soluble in dilute alkalies and alkalie carbonates, indicating pectic acid.

SUMMARY.

1. *The composition of the cell walls of algæ reported may be summarized as follows:*

Species	Outer Layer	Middle Layer	Inner Layer	Remarks
Vaucheria geminata	Pectose		Cellulose	Pectic layer difficultly permeable to copper and iodine reagents
Cladophora glomerata	Chitin	Pectose	Cellulose	Chitin very difficultly permeable to copper and iodine reagents
Oedogonium irregulare young filaments of 1-2 cells	Chitin		Pectose	Chitin very difficultly permeable to copper and iodine reagents
Oedogonium irregulare (older cells)	Chitin	Pectose	Cellulose	Chitin very difficultly permeable to copper and iodine reagents
Oedogonium crassum amplum	Unidentified	Pectose	Cellulose	Unidentified substance difficultly permeable to copper and iodine reagents
Spirogyra sp.	Pectose		Cellulose	
Zygnema cruciatum	Pectic acid (sheath) mucilaginous	Pectose	Cellulose	
Draparnaldia (main filament)	Pectic acid and pectose mucilaginous sheath	Pectose	Cellulose	

2. In all the forms studied the cell walls are composed of at least two layers, an inner one of cellulose and an outer one of pectose or of chitin. When chitin is present, pectose forms a middle layer of the cell wall.

3. In some forms, as in Vaucheria and Draparnaldia, the pectose is difficultly permeable to a solution of copper-oxide ammonia and iodine-potassium-iodide.

4. In other forms, *Cladophora glomerate* and *Oedogonsum irregulare*, a third layer composed of chitin is present; in *Oedogonium crassum amplum* a layer of an unidentified substance. These outermost layers surround the filament and may

be separated from it by appropriate reagents. These membranes are difficultly permeable to copper and iodine reagents until partially hydrolyzed.

5. The cellulose present in the algæ is readily soluble in copperoxide ammonia when the reagent is free to come in contact with the cellulose.

6. Mucilaginous sheaths of algæ studied are of pectic compounds, of which pectic acid predominates, with pectose in some cases.

The writer wishes to express her indebtedness to Dr. H. C. Sampson for directing the investigation; also to Mr. L. H. Tiffany for the identification of species of algæ studied.

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Received for publication March 10, 1923.

ALASKAN SPECIES OF DIPTERA OF THE GENUS HELOPHILUS, WITH NOTES ON OTHERS.

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It appears that several of the species of Helophilus now known from America are considered in literature under two or more names, due mainly to the introduction of a number of European species which have been named after reaching this country, or to giving separate names to European and American specimens of species which are circumpolar as many insects are, especially in high latitudes.

Most attention has been given in this consideration to the species which have a shining stripe on the face and all such species, known to me from the Nearctic fauna, are included in the key given below, while only a few of the dozen or more of the other section of the genus are treated. Notes are given on a number of species not considered in the key in order to record some observations that may be of use to future students of the group.

Much of my material was collected in Alaska by the Katmai Expeditions of the National Geographic Society. Seven species belonging to the genus were procured in series, and pains were taken to associate the sexes, and to note such variations as were presented by each species in a study of large numbers in the field.

Additional material was furnished by Chas. W. Johnson and special interest attaches to this since it was collected in eastern localities and furnished the opportunity for a comparative study of specimens from New Foundland, Labrador and Northern Maine with my specimens from Alaska. Material in various museums was studied and the results used in the conclusions which follow. The Dr. Loew collection and the cabinet of Dr. R. C. Osburn have proven interesting, as they present for study specimens from Greenland and Labrador from whence Otto Fabricius, Staeger and Loew procured their type material. Chas. L. Fluke has aided me in getting information in regard to the type of Helophilus bruesi Graenicher. He agrees that it is a synonym of borealis.

The abdominal coloration of species of Helophilus with a shining facial stripe is much the same in all. Thus groenlandicus, which was one of the first American species described, may be considered typical. The ground color is clear black, with an interrupted band of different color on each of segments two, three and four. Segment two has a rather large triangular yellow marking on either side with the inner angles distinctly gray. Segment three has a small yellow spot, somewhat variable in size, at each anterior corner and proceeding inward from it a conspicuous narrow gray marking, which does not meet its fellow of the opposite side. Segment four has the gray markings similar, but does not have any yellow whatever. Wherever the color is yellow the black is replaced, but the gray usually is over black and can be rubbed off quite easily, leaving black in its place. In at least two forms described, dychei Williston and bruesi Graenicher, the gray abdominal markings are omitted in nature, but the yellow and black persist in typical arrangement.

1. Face, between oral margin and base of antennæ, with a shining stripe devoid of pile or pollen....2
   Face without such a stripe, entirely pollinose....8
2. Facial stripe yellow or at most brown....3
   Facial stripe deep shining black....4
3. First two segments of antenna black, front of female black pilose only near the ocelli, front of male broad....*trivittatus* Fabr.
   First two segments of antenna reddish, front of female black pilose, front of male narrow....*similis* Macquart
4. All segments of all the tarsi black....5
   At least one basal segment of each middle tarsus yellow for its entire length..6
5. Thoracic stripes more or less irregular, usually not distinct, two median ones usually reaching only to the posterior fourth of the mesonotum. Thorax long yellow pilose, very rarely some black pile on the scutellum. Abdomen with the black almost wholly shining, fourth segment entirely black or with a narrow, more or less obsolete, interrupted, gray-pollinose band. *borealis* Staeger.
   Thoracic stripes regular, distinct, two median ones reaching very near to the scutellum. Thorax with rather short pile which regularly is black on the disc of the scutellum, abdomen with the black color largely opaque, shining only near the incisures, fourth segment black with a distinct interrupted, gray-pollinose band....*groenlandicus* O. Fabr.
6. Hind tibia with the yellow color more extensive than the black, hind femur yellow on nearly the apical third....*pendulus* Linneaus.
   Hind tibia with the black color plainly more extensive than the yellow, hind femur with a yellow preapical band not over one-fifth as wide as the length of the femur....7
7. Abdominal segments three and four with the posterior margins orange, thoracic stripes prominent and uniform....*hybridus* Linneaus.
   Abdominal segments three and four with the posterior margins shining black, thoracic stripe narrow, broken or varying in width on posterior half. *obscurus* Loew.

8. Male abdomen with a yellow elongate spot, triangular in form, on each side of the second segment, female abdomen with elongate, curved, gray spots on either side of third and fourth segments not in contact with the lateral margins.....*bilinearis* Williston.
Both sexes with interrupted cross-bands on each of abdominal segments two, three and four, in contact with the lateral margins.....9
9. Female abdomen with the posterior margins of segments three and four gray pollinose, male hind femora black at base.....*lunulatus* Meigen.
Female abdomen with posterior margins of segments three and four shining, male hind femora yellow at base.....*hamatus* Loew.

Helophilus borealis Staeger.

In the Canadian Entomologist for 1897, Hunter published a description of *Helophilus dychei* from Williston's manuscript. The type specimens were procured at Sitka, Alaska, by Prof. L. L. Dyche. I found the same species very plentiful at Kodiak and at Savonoski and procured a hundred or more specimens, fifty of which were pinned and kept for future study. In pinning these specimens all variations observed were selected and as the species proved to be a variable one the series furnishes an interesting study. The smallest specimen is nine millimeters in total length, while the largest one is nearly fifteen millimeters. About two-thirds of the specimens show no gray markings on abdominal segments two, three and four, while the other third show these markings plainly. The thoracic stripes are somewhat more prominent in some specimens than others, although these stripes are not prominent in any of the specimens. Even there is variation in the face which in some specimens is more prominent than in others.

Like many northern species, we would expect naturally that this one would extend entirely across the American Continent, and such is the case, for specimens from northern Maine and Mount Desert, Maine, as well as from some eastern localities further north, cannot be separated by any constant characters when long series are considered. In comparing Johnson's material of fifteen specimens above mentioned, labelled glacialis, borealis and dychei, with Alaskan material, it was possible to duplicate from the latter every specimen included in the former.

Both *Helophilus borealis* Staeger and *Helophilus glacialis* Loew are fully characterized in Williston's Synopsis of North American Syrphidæ and fully compared with *Helophilus groenlandicus* Otto Fabricius, but not with one another. The latter species is quite distinct and easily identified, but my

studies indicate that the former two names are synonyms. They are applicable to specimens with gray bands on the third and fourth abdominal segments, while *Helophilus dychei* Williston and *Helophilus bruesi* Graenicher refer to those specimens that lack these gray bands. As has been stated, some specimens show gray abdominal bands and some do not. In a series of six selected for the purpose these bands are prominent on number one and gradually become less evident until in number six they are wholly lacking, even with high magnification, yet otherwise all the specimens are of the same size, appearance and coloration.

Since borealis is the oldest name, it stands as the name of the species and *glacialis* Loew, *dychei* Williston, *bruesi* Graenicher, and I believe *androclus* Walker are synonyms of it.

The species has a wide distribution in North America, but I can find no record which would indicate that it is a member of the European fauna. It has been reported from Greenland, Labrador, New Foundland, Maine, Canada, Colorado, New Mexico and Wisconsin. In the southern parts of its range it occurs at high altitudes.

Helophilus groenlandicus Otto Fabricius.

Helophilus groenlandicus is very easily separated from borealis when specimens are at hand, but it is rather difficult to write out directions for the purpose. By using the accompanying key carefully the student should obtain the proper result. As it was named at an early date from Greenland, and as it occurs also in Northern European countries, no great surprise attaches to the fact that it has been given at least two additional names by old world dipterists. In Kertesz, Catalogue, *Helophilus arcticus* Zetterstedt and *bilineatus* Curtis are accepted synonyms, while Williston believes that *latro* Walker refers to the same species. Besides there is no very good reason for excluding *chalepus* Walker from the same synonymy.

Common at Savonoski, Alaska, where I observed many specimens and pinned nearly forty. I have seen specimens from Isle Royale, Michigan, Labrador, and New Foundland. Others have reported it from Greenland, Lapland and Europe. Specimens that I have studied are very uniform in coloration, in which respect it differs from the previous species.

Helophilus obscurus Loew.

Abundant at several stations along Naknek Lake, Alaska, in July, 1919. More than a score of specimens taken. The hind femur is nearly all black, only a narrow, more or less obscure preapical band yellowish. Median thoracic stripes more or less irregular and broken, an interrupted band on each of abdominal segments two, three and four. Fluke gives a full description of the species in his Syrphidæ of Wisconsin. Specimens are at hand also from Chippewa and Alger counties, Michigan, belonging to the University of Michigan Museum, and from Orillia, Ontario. It has been reported from Colorado, Wisconsin and Wyoming.

Helophilus pendulus Linn.

Plentiful at Savonoski, Alaska, in July and August, 1919. It is a common European species, but has not been reported from America heretofore. My specimens were compared with European examples in the United States National Museum and agree perfectly, except there is not so much black on the apical third of the hind tibia in Alaskan specimens. Somewhat similar to *obscurus*, but the thoracic stripes are very distinct, the hind femur has the apical third yellow and the hind tibia is more than half yellow. Kertesz' Catalogue gives *praecox* Rossi, *similis* Curtis, *trilenva* Harris, *trilineata* Harris and *trivittata* Meigen, all names given in Europe, as synonyms.

Helophilus hybridus Loew.

Very much of the appearance of *Helophilus similis* Macquart, but known at once from that species by the shining black stripe on the face. First recognized from North America in 1847 by Macquart when he described *Helophilus novae-scotiae* from the country after which it was named. *Helophilus latitarsis* Hunter, described in the Canadian Entomologist for 1897, from Minnesota, constitutes the second published record for America for Hunter's name is surely a synonym.

The material of hybridus before me consists of five specimens, a female from Caledonia, North Dakota, July 30, 1918, and a male and female from Banff, Alberta, July 17, 1902, R. C. Osburn, collector, a male from Fargo, North Dakota, May 27, 1917, P. W. Fattig, collector, and a male from Turtle Mountains, North Dakota, August 6, 1920, T. H. Hubbell, collector.

Helophilus trivittatus Fabricius.

This European species has been reported from the city of Mexico and Verrall published the opinion that *Helophilus latifrons* Loew, which has long been considered peculiar to America is a synonym, but later Becker announced the opinion that the two are separate.

When it is considered that a long series of specimens shows variations and also that so many related species are common to Europe and America, there are good reasons for accepting Verrall's conclusion, and his view is strengthened when one compares the specimens and descriptions available.

Kertesz in his catalogue, following Verrall, places *latifrons* Loew in synonymy with *trivittatus* Fabricius and also includes the European names *camporum* Mg. and *parallela* Harris. Hunter reports *latifrons* from Cook Inlet, Alaska, so it occurs over a wide range in America.

Helophilus similis Macquart.

The shining facial stripe is yellow or brown, the front in the male is narrow, in the female wide and almost wholly black pilose. Widely distributed over North America, but not known from Europe. Chas. R. Jones records it from England, but he writes that this is an error. Walker's names *decisa* and *fasciata* and Jaennicke's *susurrans* are admitted as synonyms in all recent lists.

Helophilus lunulatus Meigen.

The species of the genus *Helophilus* closely related to *lunulatus* are difficult to separate. The sexes of each species are different, and often it is the case that one sex or the other of two species has fairly good distinguishing characters, but the opposite sex practically is without anything of the kind. Coquillett definitely reported *lunulatus* from Alaska in 1900. Before that date it was considered strictly European, but since it has been mentioned in several local lists and from various parts of the United States. In every case descriptions in this group of species in North America have been taken from a single specimen. Loew described *hamatus* and Hunter *pilosus* from females, and Williston had the male when he described

bilinearis. Because of this, one cannot depend fully upon many of the comparisons that have been made up to the present.

Both Verrall and Lundbeck have furnished full descriptions of both sexes of *lunulatus* and have pointed out that in the female the hind margins of the abdominal segments are covered by gray pollinose bands, while the descriptions of both *hamatus* and *pilosus* mention these same parts as shining. Among my material I find no specimens that show the gray posterior bands on the abdominal segments, except a series of females taken at Savonoski, Alaska. These with the males associated with them in the field, answer the description of *lunulatus* in detail and I believe should be considered as that species.

Almost all the various species near *lunulatus* appear on the wing in early spring. I have taken what I consider *bilinearis* from spring beauty, *Claytonia virginica*, which is a well known early spring flower. Specimens of other species taken in Ohio, Indiana, Michigan and Wisconsin bear date of early May. It appears that they are not so rare if looked for at the proper season. Since there are so few specimens in the collections of the country, some careful early spring collecting and field observations are very much needed.

Helophilus laetus Loew.

Loew described four nearly related species of *Helophilus* in succession in Centuria IV. Since *laetus* is the commonest species the group is often known as the *laetus* group. It is interesting to note that the ocelli are practically as remote from each other as in *Asemosyrphus*, a genus which has been split off from *Helophilus* mainly on account of that character. These species all were described from the female sex and from a limited number of specimens, consequently they have given dipterists more or less trouble and even doubt has been expressed occasionally as to the distinctness of some of them. At last it has been possible to associate the sexes of all four and establish their validity. Practically every published list of Syrphidæ in the country records *laetus*, so it has a wide distribution. Loew's description must not be taken too literally, however, for a series of specimens shows quite a range of variations in color markings of legs and abdomen. Hunter finds by a study of the type that *Helophilus aureopilus* Towns is a synonym..

Helophilus divisus Loew.

On page 138 of Volume 29 of the Canadian Entomologist, Dr. W. D. Hunter has characterized this species. The male, although quite variable in coloration of body and legs, may be known among American species of its genus by the prominent tubercle on the under side, near the base of each hind femur. *Helophilus frutetorum* Fabr. of Europe, has even a more prominent tubercle similarly located. In a series of six males, no two have the body markings alike. There is variation also in a series of four females. Loew described a female and a translation of his description is given in Williston's synopsis. The sexes agree in possessing a narrow median pollinose mid-dorsal thoracic stripe, attenuated at both ends and dividing the median black thoracic stripe. From profile view the face is short and very little excavated beneath the antennæ. Specimens from Sandusky, Kent, Columbus and Medina, Ohio; Westville, New Jersey; Winona Lake, Ind.; Lake City, Florida, and Berrien County, Michigan. Loew's type was taken in the District of Columbia.

There is good reason for considering *Helophilus flavifacies* Bigot, a synonym of *divisus*. Bigot's specimen was taken in Maryland, and I have a specimen which answers his description in detail.

Helophilus integer Loew.

The male of this species may be known, so far as my specimens show, by the front being entirely bright yellow haired and plainly narrower above than in *divisus*, *laetus* and *obsoletus*, the only species with which it is easily confused. The female corresponds with the male in having very bright yellow thoracic stripes, but the front is extensively black pilose. It is likely a long series of specimens would show much variation in the color markings of legs and abdomen. Specimens from Massachusetts and New Jersey.

Helophilus obsoletus Loew.

Both sexes may be known by the obsolete median thoracic stripes and the slender hind femora. Loew described the female and the description is translated by Williston for his synopsis. What I consider the male of *obsoletus* is in the University of

Michigan Museum from Isle Royale. The upper half of the front is black and black pilose. The abdomen is largely black with a widely interrupted yellow band on each of segments two and three and an interrupted gray band on four. Each of segments three and four also have a gray mid-dorsal triangle posteriorly and both of these segments have something of a yellow posterior margin in connection with the triangle; femora black, yellow apically, front tibia yellow basally and blackish on apical half, front tarsi and middle tibiæ and tarsi largely yellow, hind tibiæ black, each with a narrow basal and broader median band yellow, hind tarsi mostly black on superior side and mostly yellow otherwise. I doubt if color markings of legs and abdomen are constant enough in a series of specimens to be dependable for specific distinction. Three females from Whitefish Point, Michigan, collected by McAlpin and Andrews, and a male from Isle Royale extend the known distribution of *obsoletus* much southward for the only published record heretofore gives the species as coming from Fort Resolution, Hudson Bay Territory.

Received for publication March 15, 1923.

A USEFUL CAGE FOR THE REARING OF SMALL INSECTS ON GROWING PLANTS.*

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In the rearing of aphids and their parasites, and scale insects for the past two years, the writer has found a specially designed cage of sheet-celluloid very satisfactory. Such a cage was made necessary from the unsatisfactory use of the standard lantern globe cages for rearing aphids, and other larger screen cages in common use for rearing insects on growing plants.

While sheet-celluloid has long been used by entomologists in various ways for rearing cages, chiefly to enclose portions of plants outdoors, so far as the writer has been able to learn, nothing has been published regarding it. Some time ago Mr. J. J. Davis, of Perdue University, who has been engaged in collecting data for a book on rearing methods, was asked to verify this assumption. He replied that his records disclosed no publications on the use of celluloid in the construction of rearing cages, although they had used it in Indiana for some time; but not the type of cage the writer had designed. Without claiming any originality for the idea for the use of this material in rearing cages, the writer believes it desirable to place on record his experience with it; especially in the type of cage discussed below, which he believes to be different from those in common use.

Any cage for rearing insects under normal, natural conditions, must provide for the same conditions of light, temperature and moisture within the cage as those which appear in the surrounding outside habitat; or as near these conditions as possible. In other words, the cage must allow for a free circulation of air, permit the entrance of the maximum amount of light, and not cause a condensation of heat or moisture. Still another feature bears considerable importance when dealing with small insects, and that is the absence of any cracks or niches in which the insect may hide or escape.

* Contribution No. 44 from Dept. of Forest Entomology, New York State College of Forestry, Syracuse, N. Y.

Lantern globes, so commonly employed in rearing aphids and other small insects, were extensively used in the above rearings; but under conditions of direct sunlight and higher temperature so often encountered during the spring and summer months, they were found to overheat and "sweat" so badly as to be practically useless. In casting about for a cage that would remedy the faults found in glass and still possess the other desirable features, one was constructed out of sheet celluloid, which satisfied the requirements.

The simplest and most suitable form of cage is the cylindrical type made by bending together the edges of a rectangular piece of celluloid and sealing them with 95% alcohol.† Ventilation holes of any size and position on the sheet, may be cut in before bending. After the edges are sealed together and the cylinder is formed, the ventilation openings and one end opening are covered with fine cheese-cloth or voile, shellaced on the celluloid around the edges of the openings. The cage is now ready to be placed over the plant, usually a small one in a pot. In order to hold the cage securely in position and provide a smooth white surface on the bottom of the enclosure about the plant, melted paraffine is poured on the soil around the plant, and the open end of the cage set down into it after the edges have first been given a thin coat of vaseline to prevent the adherence of the paraffine. Thus treated the cage may be lifted free when the paraffine cools, leaving a smooth tight groove into which the edges just fit and prevent the escape of any insect when the cage is in place.

In addition to the type of cage described above, the writer has found sheet celluloid a very satisfactory material with which to construct exceptionally small cages for a portion of a plant like a small twig, or a part of the surface of a leaf or fruit on which it is desirable to confine small sedentary insects such as scales and aphids. In these small cages, ventilation may be secured by punching small holes in the celluloid with a fine needle. Shellac was found useful in joining together the sharp edges of the cage and sticking it to the plant surface. This material soon drys, with a hard surface so that it will not entangle the insects, and yet remains soft enough to prevent cracking and breaking apart.

†Suggested by Mr. Larrimer of Indiana.

Cages thus constructed from sheet celluloid possess the following desirable features:

1. They are as transparent as glass, and do not "sweat" or concentrate heat like the glass cages.

2. With this material they can be made any size and shape desired.

3. They may be ventilated by cutting any number and size of openings in the side.

4. The completed cage is very neat and smooth within, making it easy to observe specimens at all times.

5. Entry for the introduction or removal of material is easily made by tearing back a corner of the cloth ventilator, or lifting up the cage from its paraffine base.

The undesirable features are:

1. Cost in material and time for making.

2. Possible deterioration on exposure to the elements. This defect was called to the writer's attention by Mr. J. J. Davis in a private communication. Automobile owners also complain of this rotting of the celluloid in their curtains. However, in the writer's experience with the material used in rearing cages, often under most unfavorable conditions of heat and moisture, he has found no such deterioration.

These many advantages possessed by celluloid have caused the writer to regard it with much favor as a material for solving the difficult problems of a suitable cage for small insects on growing plants. He hopes that the publication of this short article will bring the material to the attention of others who may have overlooked its great usefulness in cage construction.

Recieved for Publication, January, 12, 1923

SOME NOTES ON AMERICAN TABANIDÆ WITH THE DESCRIPTION OF A NEW SPECIES FROM AFRICA

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Genus Pronopes Loew.

This genus was described by Loew in 1857 for the reception of a single species, nigricans, which he described from a single male specimen from the Cape of Good Hope. A little later in his Dipterous Fauna of South Africa he further characterized the genus and published several drawings. I am able to find no further record of additional specimens, but the genus has been mentioned in several publications since.

The genus is characterized by the male having the eyes widely separated and long pilose; body short and wide; ocelli present; third segment of the antenna of five subdivisions of which the first is large and ovoid; spines at the end of the hind tibia small and rather easily overlooked.

Recently I received from Dr. A. L. Melander, two males and a female collected by Dr. Brauns at Willowmore, South Africa, January 20, 1904. The female appears quite different from the males on account of being much larger and more robust and differently colored but agrees exactly in the characters enumerated for the genus above.

Pronopes nigricans Loew is described as black with grayish black wings with blackish nerves arranged as in the genus Tabanus. The specimens before me, although they belong to the genus without doubt, appear to belong to a different species.

Pronopes flavipes n. sp.

Male, thorax mainly black, gray pollinose and pilose, wings yellowish hyaline, legs yellow, blackish near the joints, abdomen yellow. Length seven millimeters.

Female, thorax, wings and legs as in the male, abdomen dark with conspicuous light posterior margins to the segments. Length eight millimeters.

Male. Face and front nearly as wide as either eye, gray pollinose, nearly black pilose, pile at vertex abundant and long, ocelli prominent,

located as is usual for females in the subfamily; antenna dark short first segment enlarged, rounded, second segment much smaller but rounded, third segment of five subdivisions of which the basal is much the largest evenly convex above and slightly convex below. Remaining four rings small, apical ones narrowed; palpi very small, yellow, proboscis short black; eyes sparsely long pilose. Thorax dark, gray pollinose and rather long pilose, a narrow shining middorsal stripe, slightly widening towards the scutellum which is of the same color as the stripe on the disc, but the margin is gray pollinose. Wings yellowish hyaline, small stigma dark, venation normal, anal cell closed, all the posterior cells open, no stump on the anterior branch of the third vein, legs yellow, knees, tips of tibiæ and apical tarsal segments nearly black. Abdomen mostly bright yellow, first segment very largely black below and above, but sides above broadly yellow, second segment with a large black middorsal spot. Extreme tip of abdomen slightly infuscated. Actual width of abdomen at widest place about two and one-half millimeters. Actual width of front at narrowest point more than one-half millimeter.

Female. Exactly like the male except in the following particulars: Front wider than either eye, actual width at narrowest place one millimeter. Abdomen dorsally dark colored, first segment with a narrow, somewhat irregular posterior light border, second segment dark at base, posterior light border about one-third as wide as the length of its segment, expanded into a triangle at middle, remaining segments with the posterior gray borders wide and beyond the third including most of the segments. Venter yellowish gray pollinose all over.

Type male, allotype female and one paratype male from Willowmore, Capland, January 20, 1904, collected by Dr. Brauns.

Tabanus equalis n. name.

For Tabanus uniformis Hine, Ohio Journal of Science, Volume 17, page 270, 1917. There is an earlier Tabanus uniformis by Ricardo, Records Indian Museum, Volume 4, page 218, 1911.

Chrysops mœrens Walker.

Walker described the species from Nova Scotia. Chrysops æstuans Vander Wulp named from Wisconsin is considered a synonym. Recent work by various collectors has extended the known range nearly to the Pacific Coast and it should have been included in my paper on Tabanidæ of Western United States and Canada. Some Western specimens, although not all, are unusually large, but specific characters agree with those of Eastern specimens.

The species has been taken at Hermiston, Oregon and Lowden, Washington, by Melander; at Lake Paha, Washington, by Shannon; at Rittsville, Washington, by Lane, and at Grand Coulee, Washington, collector not given.

Received for Publication August 15, 1923.

A DUCK WITH WEBLESS FEET.

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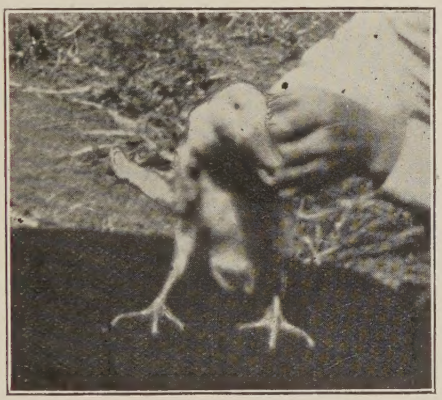
During the summer of 1921 there was hatched on the farm of Mr. Louis Weiss, near Morganville, Kansas, a duck which appeared normal in every respect except that the feet had no indication of a web. The owner had a flock of over fifty white ducks of various ages, all having the normal webbed feet except the duckling under consideration.



The toes of the duck are rather long and the prominent web is apparently an aid in walking, as well as swimming, since this mutant duck showed some difficulty in running as compared with the normal individuals. When placed in a tank, it floated about, kicking its feet violently in the water, but made rather slow progress as compared with a normal, web-footed companion.

The duck was a female and grew to maturity, but died at the development of her first egg, which was broken in the oviduct. The writer had planned to carry on breeding experiments to determine the true nature of this remarkable character. Unfortunately, not even the feet were preserved. The owner of the duck, Miss Idella Weiss, and other members of the family reported that this duck developed exceedingly

long claws, over two inches long, which interfered greatly with walking. The writer did not see the animal after the duckling stage, but there is no question as to the reliability of the reports on the long-clawed character. It is to be hoped that if a similar mutation appears at some other time or place the animal will survive, that test may be made to determine the hereditary peculiarities of the character.

The accompanying photograph, although made with a "Brownie" camera, shows the nature of the feet fairly well. There is less web between the toes than in an ordinary chicken. This mutation appealed to the writer as an unusual oddity, for here is a bird that lost, at a single jump, one of the characteristics essential to its normal mode of life and instincts.